2266-Pos Board B403
Generation of Differentially Modified Microtubules using in vitro Enzymatic Approaches
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Tubulin, the building block of microtubules, is subject to chemically diverse and evolutionarily conserved post-translational modifications that mark microtubules for specific functions in the cell. Here we describe in vitro methods for generating homogeneous acetylated, glutamylated, or tyrosinated tubulin and microtubules using recombinantly expressed and purified modification enzymes. The generation of differentially modified microtubules now enables a mechanistic dissection of the effects of tubulin post-translational modifications on the dynamics and mechanical properties of microtubules as well as the behavior of motors and microtubule associated proteins.

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A Comparison of the Conformational Changes of Tau Isoforms in the Tau-Tubulin Complex
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Tau is an intrinsically disordered protein found in the axons of neurons, where it functions to maintain microtubules and stabilize microtubule growth. It is present in the human nervous system as six differentially spliced isoforms, most noticeably half of which possess four imperfect repeats (4R) within the microtubule binding region (MTBR) and the others possess three of these repeats (3R). For poorly understood reasons, tau can form intracellular aggregates known as neurofibrillary tangles (NFTs) which have been implicated in the pathology of Alzheimer's disease and other tauopathies. This aggregation of tau in disease is thought to be precipitated by altered interactions between tau and tubulin (or microtubules). Previous studies indicate that some tauopathies possess isoform-specific aggregates, leading us to hypothesize that differences in microtubule binding between isoforms may be important to understanding the isoform-specific transition to aggregation. To investigate this, we determined the average conformational changes of tau 3R and 4R isoforms upon binding to tubulin via single molecule Förster resonance energy transfer (smFRET). Our constructs were labeled at several sets of residues within the MTBR with donor and acceptor fluorophores to elucidate the changes in residue distance between tau's solution conformation and its tubulin-bound conformation. The results provide insight into differences in the topological features of the tubulin-bound isoforms. Moreover, they may also elucidate mechanisms of association and dissociation of tau to microtubules, relevant to the initiation of aggregation.

2268-Pos Board B405
Analyzing the Frequency of Thermally Fluctuating Segments of Microtubules
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Taxol is a drug used to treat cancer by stabilizing microtubules. The purpose of this research is to understand and explain how Taxol stabilizes microtubules and build a foundation upon which new discoveries involving cancer research can be made. We analyzed if Taxol affects the vibrational modes of microtubules by determining a frequency of Taxol-stabilized microtubules. Microtubules are grown, imaged, and analyzed by measuring the change of angle in radians of the end segments at 83ms intervals. The results depicted a sinusoidal movement of the end segment of the microtubule. From this, we found the resonant frequency by taking the Fourier Transform of the data and analyzing where the maximum peak occurred. The smaller peaks in the transform may be a result of the surrounding solution or internal fluctuations of the microtubule. We interpreted a 10.2 μm microtubule to have a frequency of 0.96 Hz. The process is repeated with microtubules of similar lengths, incubated with Taxol. We compared the resonant frequencies of the various lengths of microtubules and observed that there is a relationship between the length of a microtubule and its fundamental resonant frequency. The trend shows that as length of a microtubule increases, the fundamental frequency decreases.

Cytoskeletal Assemblies and Dynamics

2269-Pos Board B406
Effects of Added Divalent Counterions on the Properties and Behaviors of Microtubule Filaments
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Microtubules are polymeric cytoskeletal filaments that define the shape of eukaryotic cells and are widely involved in intracellular active transport. Physiological regulation of microtubule mechanics and dynamics may be achieved through electrostatics based on their strong polyelectrolyte nature. Here, we report on the effects of counterions on microtubules at concentrations below the like-charge bundling phase boundary. We first show that the persistence length (Lp) is significantly increased in the presence of physiologically relevant amounts of certain divalent salts (Mg^{2+}, Sr^{2+}, and Ba^{2+}). These observations are counter to theoretical expectations and experimental observations in similar systems where biological rod-like polyelectrolytes (e.g., DNA) are reported to present lower Lp values due to counterion-induced condensation. The increase in microtubule LP was attributed to screened coulomb interactions between the filament surface and the highly negatively charged C-terminal tails. Suppression of depolymerization was also observed in the presence of Ba^{2+} and in the absence of stabilization agents (e.g., paclitaxel). The observed correlation between structural stability and mechanical rigidity is consistent with prior work involving MAPs, which also affect dynamics through interaction with the C-terminal tails. Lastly, the counterion-induced increase in Lp also significantly affected the characteristics of kinesin-transport. Here the path trajectories of microtubules in the gliding motility assay transition from highly dispersed transport to deterministic transport following the addition of divalent ions. Overall these results establish a novel mechanism by which microtubules dynamics, mechanics, and interaction with molecular motors may be regulated by physiologically relevant concentrations of divalent salts.
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2270-Pos Board B407
Spatio-Temporal Model for Silencing of the Mitotic Spindle Assembly Checkpoint
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The spindle assembly checkpoint arrests mitotic progression until each kinetochore secures a stable attachment to the spindle. Despite fluctuating noise, this checkpoint remains robust and remarkably sensitive to even a single unattached kinetochore among many attached kinetochores; moreover, the checkpoint is silenced only after the final kinetochore-spindle attachment. Experimental observations showed that checkpoint components stream from attached kinetochores along microtubules toward spindle poles. Here, we incorporate this streaming behavior into a theoretical model that accounts for the robustness of checkpoint silencing. Poleward streams are integrated at spindle poles, but are diverted by any unattached kinetochore; consequently, accumulation of checkpoint components at spindle poles increases markedly only when every kinetochore is properly attached. This step-change robustly triggers checkpoint silencing after, and only after, the final kinetochore-spindle attachment. Our model offers a conceptual framework that highlights the role of spatiotemporal regulation in mitotic spindle checkpoint signaling and fidelity of chromosome segregation.

2271-Pos Board B408
A Bundle of Antiparallel Microtubules Connects Sister K-Fibers and Balances Forces within the Metaphase Spindle
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Chromosome segregation is driven by forces generated by motor proteins and microtubules (MTs). Although MTs are highly dynamic, the metaphase spindle can be considered as a stable state as all forces acting within it are accurately balanced. Here, we propose that a new class of spindle microtubules exists which contributes to the force map of the mitotic spindle. We named this population of MTs bridging microtubules (bMTs) as they, being a bundle